COPY

Atty Docket No. 30195-PA
PTO FAX NO.: 1-571-273-0948

Attn: Ms. Chih-Min Kam

CERTIFICATION OF FACSIMILE TRANSMISSION

I hereby certify that the following in re Serial No. 09/709,237, is being facsimile transmitted to the Patent and Trademark Office on the date shown below:

(1) Declaration of Houria I. Hassouna (6 pgs);
(2) Exhibit A (15 pgs);
(3) Exhibit B (5 pgs); and
(4) Exhibit C (5 pgs).

Please note that the signature page for the Declaration of Ms. Hassouna has been sent twice; a blank page for clarity purposes, and the signed page which has been degraded by facsimilie.

Should you have any questions, please call me.

No confirmation copy of this document is being sent separately by mail.

Number of pages being transmitted, including this page: 32

Dated: January 12, 2006

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Philip H. Coelho, et al.		
Serial No.:	09/709,237	Art Unit:	1653
		Examiner:	Ms. Chih-Min Kam
Filed:	November 10, 2000		
For:	Apparatus and Method of Preparation of Stable, Long Term Thrombin From Plasma and Thrombin Formed Thereby		

DECLARATION OF HOURIA I. HASSOUNA UNDER 37 C.F.R. SECTION 1.132 IN RESPONSE TO OFFICE ACTION MAILED SEPTEMBER 29, 2005

1. I am a Professor of Medicine and Director of the Special Coagulation Center at Michigan State University, East Lansing, Michigan. For the past 34 years, I have been involved in research pertaining to immunochemistry, blood protein chemistry, and blood coagulation diagnostics. Attached hereto as Exhibit A is my curriculum vitae. This declaration is submitted in response to the Office Action mailed September 29, 2005 ("the Office Action").

2. I have reviewed the following documents: United States patent application serial no. 09/709,237 filed on November 10, 2000 by Philip H. Coelho, et al., entitled "Apparatus and Method of Preparation of Stable, Long Term Thrombin From Plasma and Thrombin Formed Thereby;" the Office Action; the July 19, 2005 claims pending before the Office Action ("the July 19, 2005 Claims"); the Draft Amended Claims (in particular, claims 10 and 19) faxed by the Applicants to the Examiner on October 3, 2005 ("the Draft October 3, 2005 Claims," which are attached hereto as Exhibit B); and the Draft

Amended Claims (in particular, claims 10 and 19) faxed by the Applicants to the Examiner on November 2, 2005 ("the Draft November 2, 2005 Claims," which are attached hereto as Exhibit C). I am familiar with the techniques and methods described in the patent application. I am also familiar with other literature in the field and with the procedures and technology used in connection with the preparation of thrombin from blood and its use in preparing a fibrin sealant or glue.

3. Based upon my review of the above and my knowledge in this field, I believe that claims 10 and 19 as set forth in the July 19, 2005 Claims, the Draft October 3, 2005 Claims, and the Draft November 2, 2005 Claims all satisfy the written description requirement.

4. **The July 19, 2005 Claims**

I understand that in the Office Action, the Examiner was concerned that the pending independent claims 10 and 19 (in the July 19, 2005 Claims) did not comply with the written description requirement because the Examiner believed that the claimed thrombin composition could not be both "free of fibrin clots" and also contain "intact plasma" (see Office Action, pages 3-4). I do not agree with the Examiner.

I believe that claims 10 and 19 in the July 19, 2005 Claims were supported by the specification. As the Examiner correctly notes at page 3 of the Office Action, the specification describes three steps, the first two of which occur at the same time: (1) preparing a fraction enriched in prothrombin using ethanol to substantially enhance the concentration of prothrombin and to remove or denature naturally occurring ingredients in the plasma which bind, block, or interfere with or inhibit prothrombin or its activation to functional thrombin; (2) adding calcium ions to the enriched prothrombin solution and agitating the solution to convert the prothrombin to stable, long term thrombin; and (3) filtering the thrombin solution to remove particulate matter. (Specification, page 8.) The Examiner is also correct that the specification indicates that the resulting thrombin composition obtained after filtration does not contain "intact plasma." However, this point is not relevant because claims 10 and 19 do not refer to "intact plasma." It is true that the claimed thrombin composition does not contain "intact plasma," because the

filtration step has substantially reduced the proteins contained in the original plasma. (See specification, page 16, and figure 8.) As stated at page 16 of the specification:

> "Figure 8 reflects the effect of using ethanol at 13.6% and calcium chloride at .023μM to reduce proteins which alter the clot time of the thrombin as compared to the original plasma. As can be seen in this graph, the major interfering proteins are so efficiently removed, that the clotting time of the thrombin is not only enhanced, but held substantially stable and constant."

It is clear from the specification that the resulting thrombin composition does contain plasma, but does not contain the "original plasma" (referred to by the Examiner as "intact plasma"). Claims 10 and 19 set forth in the July 19, 2005 Claims do not refer to "intact plasma"; they refer to "plasma." A person reasonably skilled in the art of blood protein chemistry would understand the term "plasma" as used in these claims, in light of the specification, to indicate that portion of the original plasma remaining after filtration and removal of the various proteins, and would not be confused. In other words, "plasma" is still properly called "plasma" even after it has been depleted of some of its proteins. In fact, the concentration of various blood proteins in the plasma of humans varies substantially, but it is still referred to as "plasma."

Thus, I believe that claims 10 and 19 as set forth in the July 19, 2005 Claims satisfy the written description requirement.

5. **Draft October 3, 2005 Claims**

I also believe that claims 10 and 19 in the October 3, 2005 Claims very clearly satisfy the written description requirement. The added clause: "whereby the plasma has been substantially depleted of the particulate matter sequestered by filtration," expressly states what is understood by a person who is reasonably skilled in the art, as set forth in paragraph 4 above.

6. **Draft November 2, 2005 Claims**

I also understand that the Examiner is concerned about the range of ethanol as set forth in the Draft November 2, 2005 Claims. I understand that the Examiner is concerned that claims 10 and 19, which specify an ethanol concentration of 8-18%, are not supported by the specification. I respectfully disagree with the Examiner.

The specification describes the utility of the range of 8-18% ethanol concentration at page 16 and is shown in figure 5. The specification states, at page 16:

> "Turning to figure 5, a graph is shown which illustrates how ethanol concentrations alter the life span of fast clotting thrombin where the calcium chloride content is held constant at .023μM. Note that at approximately 13.6% ethanol, its lifespan is shown to have been optimized and extend at least 240 minutes while its clotting time is substantially constant at under 5 seconds. The range between 8% and 18%, however, has utility."

Figure 5 shows that at 8% ethanol concentration, the lifespan of thrombin that will clot in less than 25 seconds is 210 minutes, while at 18% ethanol concentration, the lifespan of thrombin that clots in less than 10 seconds is 210 minutes. This supports the language of claims 10 and 19.

I understand that the Examiner is concerned that the reference to "plasma" in claims 10 and 19 as set forth in the November 2, 2005 Claims is not supported by the specification for the same reasons set forth in the Office Action (i.e., that the "plasma" in the thrombin composition after the filtration step is not the same as the "intact plasma" from which the composition is made). Again, as I stated in paragraph 3 above, a person skilled in the art would understand that these claims, in light of the specification, are not referring to the "original plasma," but to the plasma remaining after filtration. And again, "plasma" is the proper word to describe the liquid blood remaining after some of the proteins have been removed.

Lastly, I understand that the Examiner is concerned that claims 10 and 19 as set forth in the November 2, 2005 Claims do not satisfy the written description requirement because the Applicants' data shown in figure 8 utilized 13.6% ethanol, not 8-18% as claimed. This is not a valid concern. To a person reasonably skilled in the art, it is understood that figure 5 refers to the same thrombin composition as was prepared using 13.6% ethanol concentration, except that either 8% or 18% ethanol concentration was used. In light of figure 5, it is clear to a person skilled in the art that the results shown in figure 8 would not be different if the ethanol concentration was in the range of 8-18%.

The proteins shown in figure 8 would still precipitate in substantially the same amount. Thus, the November 2, 2005 Claims satisfy the written description requirement.

7. I hereby state that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code.

Dated: December ___, 2005

Houria I. Hassouna

The proteins shown in figure 8 would still precipitate in substantially the same amount. Thus, the November 2, 2005 Claims satisfy the written description requirement.

7. I hereby state that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code.

Dated: December 23, 2005

Houria I. Hassouna

Houria I. Hassouna

EXHIBIT A

Exhibit A

CURRICULUM VITAE

Houria I. Hassouna, MB, B.Ch., Ph.D.

Title: Professor, Medicine , Department of Medicine, College of Human Medicine, Michigan State University — 1980 - Present

Director, Special Coagulation Center,

Certification # CLIA 23DO 723132 Department of Medicine, College of Human Medicine, Michigan State University — 1984 - Present

Degrees: M.D., B.Ch. with honors, Faculty of Medicine, Cairo University, Cairo, Egypt — 1965

Ph.D., Rockefeller University — 1975

Residency Status:

Exchange Visitor	1969 - 1971
Exchange Visitor	1972 - 1977
Permanent Resident	1977 - Present

Citizenship: Egypt

Tenure Granted: January 1, 1988

Home Address: 3 Lakeside Court, Grosse Pointe, MI 48230 — Telephone: (313) 343-0715

Office Address: Department of Medicine, B-214 Clinical Center, East Lansing, MI 48824-1313 — Telephone: (517) 353-5080

Married (1955): Salah K. Adel, M.D., B.Ch. - DGO, D.S. - M. Chir., FACOG
Professor, Obstetrics and Gynecology, Faculty of Medicine, Cairo University;
Clinical Professor, Ob-Gyn, Wayne State University, Detroit, MI

Children: Nimetallah , Ibrahim , Shereen

Education:

Bac, es Philosophy	Lycee Francais, Cario, Egypt Metaphysics, Philosophy	June 1951
Bac. es Sciences	Lycee Francais, Cario, Egypt Biology, Physics, Chemistry, Math	October, 1951
M.B., B.Ch.	Faculty of Medicine, Cairo University, Cairo, Egypt Medicine, Surgery	December, 1964
Ph.D.	Rockefeller University New York, NY Physiology; Title of Dissertation: "Role of the Pituitary	December, 1974

Exhibit A

in Maintenance of Early Pregnancy in the Rat by Immunological and Biological Partners."

Undergraduate Medical Studies: Hammersmith Hospital, London, England Royal Infirmary Hospital, Edinburgh, Scotland Hotel Dieu, Paris, France Canton Hospital, Zurich, Switzerland		1955 - 1956
Postgraduate Studies:		
Human Histology, Human General and Special Pathology	Department of Pathology, Faculty of Medicine Cairo University, Cairo, Egypt	October, 1965 - December, 1968
Immunologic and Immunochemical Studies of Reproduction	The Rockefeller University Bio-Medical Division, New York, NY	April, 1969 - July, 1971
Immunochemical Studies of Coagulation Factors	Wayne State University Detroit, MI	October, 1973 - December, 1974

Academic and Professional Appointments:

Housewife	Cairo, Egypt	1956 - 1961
Rotating Internship	Cairo University Hospitals, Cairo, Egypt	1964 - 1965
Clinical Demonstrator, Gynecological Pathology	Department of Obstetrics and Gynecology, Faculty of Medicine, Cairo University	1965 - 1969
Postdoctoral Fellow	Biomedical Division, Rockefeller University	1969 - 1971
Research Associate	Department of Physiology, Wayne State University School of Medicine	1973 - 1974
Assistant Professor	Department of Physiology, Wayne State University School of Medicine	1975 - 1978
House Officer VI	Department of Internal Medicine, University of Michigan School of Medicine	1978 - 1979
Research Assistant Scientist	Division of Hematology and Oncology, University of Michigan School of Medicine	1979 - 1980
Assistant Professor	Department of Obstetrics and Gynecology Faculty of Medicine, University of Cairo	1979 - 19
Associate Professor	Department of Medicine and Pathology, Michigan State University	1980 - 1996
Director	Special Coagulation Center, Department of Medicine Michigan State University	1983 - Present
Professor	Department of Medicine, Michigan State University	1996 - Present

Exhibit A

Administrative Responsibilities:
Director, Special Coagulation Center
Course Director, Techniques Workshop
Course Director, Annual Penner Blood Coagulation Conference
Member North American Specialized Coagulation Laboratory Association (NASCOLA)

Membership in Scientific Societies:
The New York Academy of Sciences
American Heart Association
The International Society on Thrombosis and Haemostasis
The American Association for Advancement of Science
Michigan Research Society
Council Member American Heart Association
Central Society for Clinical Research

Appointments:
Member of Research Fellowship Committee, Michigan Heart Association
Biomedical Student Research Committee, Michigan State University
Faith and Hope Organization of Detroit, Vice President
Student Performance Committee, Michigan State University
Consultant for the Consortium for International Development, Tucson, AZ
Laboratory Committee
College-Wide Evaluation Committee
Consultant to L Vad Technology, Inc., Sinai Hospital SCIR Phase II Project

Honors and Awards:

Medical Fellowship	Rockefeller University, New York, NY
Medical Fellowship	Awarded by Population Council Department of Ob-Gyn & Physiology Wayne State University School of Medicine
Research Scientist	Ford Foundation
Elected Member	Alpha Omega Alpha Honor Society
Elected Member	Central Society for Clinical Research

Patents:

US Patent # 5,051,357: Method and Assay Using Inactivation of Factors Va and VIIIa by Activated Protein C to Diagnose Thrombic Disease or Assay for Protein C and Kit Thereof. 1991

US Patent # 5,525,477: Method for Diagnosing Blood Clotting Disorders. 1996

Visiting Professor and Seminars Presented:

Seminar and organized workshop on "Specific Effects on Antibodies on the Trophoblast" at the Max Panck Institut for Immunbiologie, Freiburg, West Germany. May, 1974

"Immunity and Disease" at Oakland College, Huntsville, AL. May, 1975

Twelfth Annual Postgraduate Course on Blood Coagulation for the Laboratory and the Clinician at the University of Michigan Medical School in Ann Arbor, MI. October, 1977

Title of talk: "Radioimmunoassay in Coagulation Testing."	
"Some Selected Aspects of the Mechanisms of Blood Clotting in Invertebrates and Mammals" at the Department of Biological Sciences, University of Idaho, Moscow, ID.	March, 1978
Department of Pathology, Loyola University Medical Center, Chicago, IL: "Immunoassays in Coagulation Testing."	March, 1979
Department of Physiology, Michigan State University, East Lansing, MI: "Overview of Coagulation."	October, 1980
Department of Pathology, Michigan State University, East Lansing, MI: "Antibody Techniques and Blood Coagulation."	April, 1981
Grand Rounds, Hutzel Hospital, Department of Thrombosis: "On Antithrombin III, the Natural Anticoagulant."	March, 1982
Faculty of Medicine, Department of Ob/Gyn, Cairo University, Cairo, Egypt: "The Role of Platelets in Hemostasis."	April, 1982
Biomedical Students Research Forum, Michigan State University, East Lansing, MI: "The Significance of Thrombosis."	September, 1982
ASMT Annual Meeting, Fort Wayne, IN: "Immunodiagnostic Approach to Coagulation Testing."	April, 1983
FIX Subcommittee of the International Society for Thrombosis and Hemostasis, Bergamo, Italy: "A Factor VIII Deficient Rabbit Model to Test Thrombogenicity of Factor IX Concentrates."	June, 1983
Michael Reese Hospital and Medical Center, Chicago, IL: "A Factor VIII Deficient Rabbit Model."	September, 1983
Department of Medicine, Pathology and Environmental Toxicology, Michigan State University, East Lansing, MI: "Protein C."	April, 1984
Owosso Memorial Hospital, Owosso, MI: "Fibrinolytic System."	April, 1984
Grand Rounds, St. Lawrence Hospital, Lansing, MI: "Bleeding in the Alcoholic."	May, 1984
The Region IV ASMT Annual Meeting, Hyatt Regency Hotel, Dearborn, MI: "Special Coagulation Testings."	September, 1984
Southwestern Michigan Area Health Education Center, Kalamazoo, MI: "Hemostasis and Thrombosis Updates: Antithrombin III and Protein C - Their Current States."	October, 1984
Grand Rounds, Bon Secouis Hospital, Grosse Pointe, MI: "Overview of Hemostasis."	October, 1984
Grosse Pointe Academy, Grosse Pointe, MI: "The Land of the Pharaohs."	November, 1984
Fifth Annual Intensive Internal Medicine Board Review Course, Providence Hospital, Department of Medicine, Detroit, MI: "Hemostasis."	March, 1985
Department of Biochemistry, Michigan State University, East Lansing, MI: "Blood Coagulation Enzymes."	March, 1985
College of Veterinary Medicine, Michigan State University, East Lansing, MI: "Decay of a Civilization."	May, 1985

Ingham Medical Center, Lansing, MI: "Laboratory Approach to Solving Bleeding Problems."	October, 1985
Visiting Professor in Internal Medicine, Hurley Medical Center, Flint, MI: "Hypercoagulable State."	December, 1985
Norton Centennial Series, The University of Louisville, Kentucky School of Medicine, Louisville, KY: "Thrombosis at the Vascular Level and Laboratory Evaluation of Accelerated Coagulation."	March, 1986
Lenawee Medical Society, Adrian, MI: "The Role of the Fibrinolytic System in Thrombosis."	March, 1986
Ingham Medical Center, Lansing, MI: "The Significance of Fibrin Split Products in the Evaluation of Intravascular Clots."	July, 1986
Ingham Medical Center, Lansing, MI: "Differential Diagnosis of a Bleeding Disorder."	January, 1987
University Grand Rounds, Michigan State University, East Lansing, MI: "Advances in Thrombolytic Therapy."	March, 1987
Grand Rounds, Owosso, MI: "Recent Advances in Thrombolytic Therapy."	April, 1987
Department of Surgery, St. Lawrence Hospital, Lansing, MI: "Heparin and Protamine Sulfate."	May, 1987
Primary Care Conference, Ingham Medical Center: "Controversies in Anticoagulation Therapy."	July, 1987
I was invited to organize and chair a workshop on "Endogenous Mechanisms in Coagulation and Anticoagulation Disorders During Shock" at the XIIth Annual Conference on Shock held in Marco Island, FL.	June, 1989
Keynote speaker on "Effect of Contraception on the Hemostatic System" at the National Family Planning and Reproductive Health Association's (NFPRHA) 17th Meeting at the Mayflower Hotel, Washington D.C.	November, 1989
Invited Speaker at the Vth Annual Venezuelan Congress of Specialized Bio Analysts, held in Puerto La Cruz, Venezuela. I gave two talks: "Blood Vessels, Platelets and Plasma Components: Their Role in the Hemostatic Process" and "Thrombosis and Conditions that Lead to Thrombotic Disorders." My photograph and a report on my talks appeared in the national newspaper.	April, 1991
Invited to organize and chair a workshop at the American Society of Medical Technology ASMT/'91, 59th Annual Meeting on "Clinical Challenges and Laboratory Diagnostic Panels for Bleeding and Thrombotic Disease" in Atlanta, GA.	June, 1991
Invited to speak at the GRAMEC Plastic Surgery Residency Academic Grand Rounds Conference "Bleeding and Thrombotic Disorders." Grand Rapids, MI	August, 1991
"Laboratory Assessment of Coagulation Disorders," Frances Warde Medical Laboratory, Ann Arbor, MI	November, 1992
Meet the Professor "Laboratory Diagnosis of Bleeding in High Risk Obstetrics"	May 1993

42nd Annual Clinical Meeting, American College of Obstetricians and Gynecologists, Washington D.C.	
"AD HOC Committee Meeting to Discuss Treatment Options for the Management of Bleeding Episodes in Adult Patients with Factor VIII Inhibitors," Chicago, IL. Two of our GMEI residents, Robert Monger, M.D. and Isabel Matheson, D.O., went with me and presented results of research they had accomplished in my lab.	November, 1993
Invited to organize and chair a Satellite Session at the IVth Biennial Meeting on Blood Coagulation and Platelet Biology: "Thrombin Functions and New Prospects in Antithrombotic Therapy." Session Entitled: "Skin Flap Autographs in a Porcine Animal Model. Enhancement of Healing Process by Autologous Fibrinogen Cryprecipitate and Fibrin Glue." Megeve (a beautiful ski village in the French Alps), France.	September, 1994
Invited Speaker at the second FASEB Conference on Thrombin, "Vascular Functions on Thrombin." Title of my talk: "Thrombin in Healing Process." Copper Mountain, CO	August, 1995
Invited Speaker at the XXIth International Congress of Pediatrics, Cairo, Egypt. I presented two papers: "Protein C Resistance" and "Effects of Platelets and Thrombin on Wound Healing."	September, 1995
Grand Rounds, Michigan State University, Department of Medicine, East Lansing, MI: "Coagulopathies as it relates to Diagnosis."	October, 1995
Round Table Discussion, ACOG 5th District Meetings, Hawaii: "Diagnosis of Fibrinogen Disorders."	November, 1995
Invited Speaker to the symposium "Therapeutic Use of Antithrombin III Concentrates in Trauma Associated with Impact, Surgical, or Burn Injuries." Title of my talk: "Antithrombin III, Characteristics and Function," Phoenix, AZ.	May, 1996
Invited to speak on "Diagnosis of the Hypercoagulable State" to the Pharmaceutical Divisions of DuPont Pharma in Wilmington, DE.	June, 1996
Organized a workshop on "Therapeutic Modalities of Platelets and Endothelial Cell Functions" for the American Society for Clinical Laboratory Sciences ACLS Annual Meeting "Winds of Challenge." Chicago, IL	August, 1996
Attended a Round Table Conference in Sardinia, Italy (by invitation only) on "Inhibitor Bypass Activity."	September, 1996
11th Annual Pharmacy Invitational Conference on Anticoagulation Therapy: title of of talk: "Emerging Issues in Hypercoagulability/Basic Science Discoveries."	December, 1996
Primary Care Update, Mercy General Health Partners. Title of talk: "Factors that Cause Stroke." Muskegon, MI	December, 1996
Department of Internal Medicine Meeting, Michigan Capital Health Care. Title of Talk: "Cerebrovascular Stroke."	February, 1997
The 3rd FASEB Conference on Thrombin and Vascular Medicine. Title of talk: "Activation of Factors V and VIII in Blood."	August, 1997
Central Society for Clinical Research. Title of talk: "Activation of Native Protein	
September, 1998	

Exhibit A

C by APC."

15th International Congress on Thrombosis, Antalya, Turkey. Title of talk: "Inactivation of Protein C by Activated Protein C (APC) in Human Plasma." October, 1998

134th Annual Scientific Meeting of the Michigan State Medical Society "Dilemmas in Diagnosis and Management of Hemorrhagic and Thrombotic Disorders." Title of talk: "A Primer on Hemostasis and Thrombosis," Dearborn, MI. November, 1999

University Grand Rounds, Michigan State University, East Lansing, MI:
March, 2000
"Fibrinolysis Enzyme System: Relationship to Bleeding and Thrombosis."

16th International Congress on Thrombosis: "Added APC in the APC-Resistance Test Activates Plasma Protein C," Porto, Portugal. May, 2000

Invited Speaker at the 28th Annual Meeting of the Japanese Society of Vascular Surgeons. "Clinical Significance of Antithrombin Agents (Hirudin and Argatroban) in Treatment of Arterial and Venous Thrombosis," and "New and Future Trends in Antithrombotic Therapy for Patients with Obstructive Arterial Diseases, Small-Caliber Arterial Prosthetic Graft, and Venous Thrombosis," Tokyo, Japan. May, 2000

Invited Speaker Hyogo Prefectural Awaji Hospita, Awaji, Japanl: May 200

A Guide for Rapid Diagnosis of Acute and Chronic/Subacute Disseminated" and Intravascular Coagulation (DIC)," Awaji, Japan.

Invited Speaker Pasteur Institute " role of exogenous factors on coagulation " June 23, 2001

Invited Speaker Penner Conference Cairo Egypt
" Proteolysis of Protein C by Activated Protein C " October 2001

Invited Speaker and Organizer Educational Program.
International Society of Hematology Cairo Egypt January 5-9; 2002
" Role of Tissue Factor Pathway in the initiation of Coagulation

Invited Speaker, World Conference on Dosing of Antiinfectives- Dosing the Magic Bulletts and the Ehrlich Symposia .organized by Professor Fritz Sorgel Nurnberg German Federal Republic Germany "APC Anti-infective Action is augmented by APC activation of Protein C"

September 9-11, 2004

Submitted Abstracts accepted as Oral presentations at Symposia and Scientific Sessions at International Meetings:

Paris, France. Vth International Congress on Haemostasis and Thrombosis. Quantitative Determination of Prothrombin and Its Fragments in Plasma and Serum. July, 1975

"Philadelphia, Pennsylvania. VIth International Congress on Thrombosis and Haemostasis. "Studies Involved in the Development July, 1977

of a Specific Radioimmunoassay for Plasma Prothrombin" - Symposium Session.

Istanbul, Turkey. International Society of Haematology. September, 1977
Immunological Characterization and Quantitation of Bovine Vitamin K Dependent Proteins" - Symposium Session.

London, England. VIIth International Congress on Thrombosis and July, 1979
Haemostasis. "Radiolabeled Antibodies to Measure Plasma Prothrombin."

Toronto, Canada. VIIIth International Congress on Thrombosis July, 1981
and Haemostasis. "Physiologic Role of Plasma Inhibitors in Inactivation and Binding of Thrombin."

Stockholm, Sweden. IXth International Congress on Thrombosis July, 1983
and Haemostasis. "A Diagnostic Assay for Protein C" and "Investigation of Activated Prothrombin Complex in a Factor VIII Deficient Rabbit Model."

San Diego, California. Xth International Congress on Thrombosis July, 1985
and Haemostasis. "Monoclonal Antibodies to Probe the Structural Homology of the Heparin Binding Site(s) on the Heparin-Dependent Blood Coagulation."

San Diego, California. Xth International Congress on Thrombosis July, 1985
and Haemostatis. "The Effect of Varying Concentrations of Heparin and Antithrombin III on the Inactivation of Thrombin in Plasma by Heparin Cofactor II."

Sydney Australia International Society on Thrombosis & Haemostasis XXth Congress
" Activation of Protein C augments APC anti-infective Action. August 6-12, 2005

Abstracts Presented at Local Meetings:

Athens, Georgia. Society for the Study of Reproduction. August, 1973
"Repeated Pregnancy Termination in the Rat with LH Antiserum"

Anaheim, California. American Heart Association, 48th November, 1975
Scientific Session. "Association of Profragment 2 of Prothrombin with Purified Ac-Globulin (Factor V)."

Chicago, Illinois. Federation of American Scientists. FASEB April, 1977
"Inhibition of Enzyme Activity of Bovine Prothrombin Molecule by Antibodies. Localization of its Antigenic Determinant Sites."

Chicago, Illinois. American Federation for Clinical Research. November, 1978
Midwest Section Meeting. "Inactivation of Prothrombin by Antibodies."

Anaheim, California. Federation of American Societies for Experimental Biology. "Characterization of Antibody Binding Sites to Antithrombin III" and "Association Kinetics of Radio-labeled Blood Clotting Ligands to Binding Agents Studied by Gel Chromatography." — April, 1980

Chicago, Illinois. American Federation for Clinical Research, Midwest Section Meeting. "On the Nature of the Bypassing Activity of the Prothrombin Complex Concentrates." — November, 1981

Abstracts Presented by Students at Local and International Meetings:

Anaheim, California. Federation of American Societies for Experimental Biology. R. Grimshaw, J. A. Penner, H.I. Hassouna: "Association Kinetics of Radiolabeled Ligands to Binding Agents Studied by Gel Chromatography." — April, 1979

Anaheim, California. Federation of American Societies for Experimental Biology. M.P. Milad, H.I. Hassouna: "Characterization of Antibody Binding Sites to Antithrombin III." — April, 1979

Toronto, Canada. VIIIth International Congress on Thrombosis and Haemostasis. M.P. Milad, H.I. Hassouna: "Clotting and Chromogenic Substrate Assays Measure Separate Thrombin Activities." — July, 1981

Toronto, Canada. VIIIth International Congress on Thrombosis and Haemostasis. R.J. Cobel-Geard, J.A. Penner, H. I. Hassouna: "Interaction of Protamine Sulfate with Thrombin." — July, 1981

Toronto, Canada. VIIIth International Congress on Thrombosis and Haemostasis. F.A. Kennedy, H.I. Hassouna, J.A. Penner, J. Schulz: "Role of Prothrombin and Factor IX in the Initiation of Thrombus Formation." — July, 1981

Chicago, Illinois. Central Society for Clinical Research. November, 1981
M.P. Milad, H.I. Hassouna: "Clotting and Amidolytic Activities of Thrombin Complexed with Antithrombin III."

Chicago, Illinois. Central Society for Clinical Research W.D. Shepard, H.I. Hassouna, J.A. Penner: "The Role of -1-Proteinase Inhibitor in the Inactivation of Factor X_a." — November, 1982.

Chicago, Illinois. 63rd Conference of Research Workers in Animal Diseases. L.G. Portnoy, H.I. Hassouna: "An Experimentally Induced Hemophilic Rabbit Model." — November, 1982

Chicago, Illinois. Central Society for Clinical Research. R. Dykstra, H.I. Hassouna, J.A. Penner: "Anticoagulant Properties of a Chemically Modified Antithrombin III." — November, 1984

Chicago, Illinois. Central Society for Clinical Research. R.J. Smith, J.A. Penner, H.I. Hassouna: "Effects of Prothrombin Enzymes on a FVIII Deficient Rabbit Model." — November, 1984

Cardiovascular Research Forum. G. Eiland, H.I. Hassouna: "Antithrombin III Monoclonal Antibodies Detect Heparin Binding Site." — November, 1984

SanDiego, California. Xth International Congress on Thrombosis and Haemostasis. C. Morgan, H. Hassouna: "The Effect of Varying Concentrations of Heparin and Antithrombin III on the Inactivation of Thrombin in Plasma by Heparin Cofactor II." — July, 1985

SanDiego, California. Xth International Congress on Thrombosis and Haemostasis. J. Barry, J. Penner, H. Hassouna: "Monocolonal Antibodies to Probe the Structural Homology of the Heparin Binding Site(s) on the Heparin-Dependent Blood Coagulation Proteins." — July, 1985

New Orleans, Louisiana. ICAAC. G.E. Stein, H.I. Hassouna, N. Mummaw, J.A. Penner: "Effect of Cefpiramide and Cefoperazone on Blood Coagulation and Platelet Function." — September, 1986

Shock Society Meeting. Circulatory Shock, Vol. 18, #4, 1986 p. 337. J.G. Hauptman, L.J. Drane, H.I. Hassouna, J.A. Penner, T.G. Bell, T.E. Emerson: "Improvement of Coagulation Variables by Antithrombin III (AT-III) Administration in Endotoxin Induced Disseminated Intravascular Coagulation (DIC) in the Dog." — June, 1986

Washington, D.C. Federation of American Society of Experimental Biology. M.E. Hogan, H.I. Hassouna, K.L. Klomparens: "Sectionable Microcarrier Beads: An Improved Method for the Preparation of Cell Monolayers for Electron Microscopy." — April, 1987

Abstracts Present by Students at Local and International Meetings 1987- 2005 : 56 abstracts
Michigan State University Biomedical NIH Research Programs 1987- 2000 : 35 trainees

Student Research Fellowship Awarded By American Heart Association of Michigan:1983-

Doctoral Students:
Margaret Hogan, MS, Ph D 1987
Christopher Quinn, DO, MS 2004
Chad Patton, MS 2001
Adam Coughlin, MS 2003
Adam Coughlin PhD 2005
Paul Nagelkirk, MS 2002
Paul Nagelkirk PhD 2005

Exhibit A

Publications:

KA Laurence and HI Hassouna. Antihormones and Their Use in Studies of Reproduction. Biol Reprod 1972; 6:422-6.

KA Laurence, CC Chang, HI Hassouna, S Badawy and K Loweitt. Effects of Antigonadotropins on Ovarian Function. Gynec Invest 1972; 2:57-64.

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WH Seegers, HI Hassouna, D Hewett-Emmett, DA Walz and TJ Andary. Prothrombin and Thrombin: Selected Aspects of Thrombin Formation, Properties, Inhibition and Immunology. Sem Throm Hemost (EF Mammen, Ed.), 1975; Vol. 1, 3.

WH Seegers, E Novoa, DA Walz, TJ Andary and HI Hassouna. Effects of Prothrombin Fragments on Thrombin, on Thrombin Formation, and Separation from Ac-Globulin (Factor V). Thromb Res 1976; 8:83-97.

WH Seegers, E Novoa, RL Henry and HI Hassouna. Relationship of "New" Vitamin K-Dependent Protein C and "Old" Autoprothrombin II-A. Thromb Res 1976; 8:543-52.

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WH Seegers, HI Hassouna and E Novoa. Immunological Aspects of Some Vitamin K-Dependent Factors and Preparation of Depleted Plasmas. Thromb Res 1977; 11:633-42.

WH Seegers, E Novoa, TJ Andary and HI Hassouna. Bovine Antithrombin III: Miscellaneous Observations. Thromb Res 1977; 11:809-815.

HI Hassouna and RE Leach. Immunological Characterization and Quantification of Bovine Vitamin K-Dependent Proteins. Bibl Haemat 1977; 44:28-38.

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WH Seegers, ME Ekstrom, CA Harper and HI Hassouna. Usefulness of New Analytical Reagants Demonstrated with Coumadin-Modified Plasma. Bibl Haemat 1977; 44: 61-7.

HI Hassouna and JA Penner. Antibody Techniques and Blood Coagulation. Sem Thromb Haemost (EF Mammen, Ed.), 1981; Vol. 7, No 2:61-111.

JA Penner and HI Hassouna. Inhibitor Disorders with Comments on Management. Proceedings of the Second International Symposium on Hemophilia Treatment, Tokyo, Japan. Kyoritsu Printing, Tokyo, 1981

MP Milad and HI Hassouna. Evaluation of Clotting and Amidolytic Activities of Alpha-Thrombin Complexed with Antithrombin III. Thromb Res 1983; 30:101-5.

RJ Cobel-Geard and HI Hassouna. Interaction of Protamine Sulfate with Thrombin. Am,Jour of Hemat 1983; 14:227-33.

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T Boenig and HI Hassouna. A Laboratory Approach to Solving a Bleeding Disorder. The Female Patient.75; 35-89 1989

HI Hassouna and G Stein. Bleeding Diatheses and Newer Cephalosporins American Journal of Hematology 878-858 1989.

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CM Patton, H Hassouna, Cjwomack. Changes in von Willebrand factor and fibrinolysis following a post exercise cool-down. Eur J Appl Physiol (2004) 92:328-333

Guest Editorships and Chapters in Books:

HI Hassouna. Contributions from Monoclonal Antibody Studies. Prothrombin and Other Vitamin K-Dependent Proteins. WH Seegers and DA Walz, Ed. CRC Press, Boca Raton, FL, 1985

HI Hassouna. Acute, Chronic Thrombosis and Disseminated Intravascular Coagulation. in Cellular Pathology. MM Sayeed, Ed. CRC Press, Boca Raton, FL, 1989.

Hematology/Oncology Clinics of North America: Coagulation Disorders I. WB Saunders Co., Philadelphia, PA, USA. October, 1992.

Hematology/Oncology Clinics of North America: Coagulation Disorders II. WB Saunders Co., Philadelphia, PA, USA. December, 1993.

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JW Fenton II, FA Ofosu, K Henrickson, DV Bresniak and HI Hassouna. Thrombin Participation in Cancers. in New Anticoagulants for the Cardiovascular patient Hanley Belfus Inc/Philadelphia ed. Roque Pifarre 1997 517-520.

Hematology/Oncology Clinics of North America: Blood Stasis and Thrombosis. WB Saunders Co., Philadelphia, PA, USA. April, 2000.

Handbook for the Basic Mechanism and the Diagnosis of Bleeding and Thrombosis Oxford Cambridge In Press

Awards and Recognition:

Teaching Awards medical students (1987, 1988, 1993, 1995, 1997,2000, 2001,2003, 2004)
Recognition awards minority medical students (1987, 1998, 2003)
Resident Teaching Award 2003
Department of Medicine outstanding Educator 2001

EXHIBIT B

Exhibit B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Philip H. Coelho, et al.	Art Unit:	1653
Serial No.:	09/709,237	Examiner:	Ms. Chih-Min Kam
Filed:	November 10, 2000		
For:	Apparatus and Method of Preparation of Stable, Long Term Thrombin From Plasma and Thrombin Formed Thereby		

DRAFT AMENDED CLAIMS

Claims 1-9 (cancelled)

Claim 10 (currently amended) - A thrombin composition free of fibrin clots, consisting essentially of:

Plasma;

Ethanol (EtOH); and

$CaCl_2$;

whereby the plasma has been substantially depleted of the particulate matter sequestered by filtration.

Claim 11 (previously presented) - The composition of claim 10 wherein ethanol is present at 13.6% by volume and $CaCl_2$ is present at 0.023 μM.

Claim 12 (withdrawn) – A method for preparing thrombin comprising:

obtaining plasma;

adding only ETOH and $CaCl_2$ to the plasma, forming a composition;

agitating the composition;

filtering the composition of particulate, thereby passing the thrombin through the filter.

Claim 13 (cancelled)

Claim 14 (previously presented) - The composition of claim 10 wherein ethanol is present at a concentration between about 8% and about 18% by volume.

Claim 15 (previously presented) - The composition of claim 10 wherein $CaCl_2$ is present at a concentration between about 0.011 μM and about 0.045 μM.

Claims 16-18 (cancelled)

Claim 19 (currently amended) - A thrombin composition free of fibrin clots, consisting essentially of:

plasma;

ethanol (EtOH); and

a source of calcium ions;

whereby the plasma has been substantially depleted of the particulate matter sequestered by filtration.

Claim 20 (previously presented) - The composition of claim 10 wherein ethanol is present at 13.6% by volume.

Claim 21 (previously presented) - The composition of claim 10 wherein $CaCl_2$ is present at 0.023 μM.

Claim 22 (previously presented) - The composition of claim 10 wherein thrombin isolated from the composition has a clotting time of about five seconds or less and is stable for more than 15 minutes.

Claim 23 (previously presented) - The composition of claim 10 wherein thrombin isolated from the composition has a clotting time of about five seconds or less and is stable for about 240 minutes or greater.

Claim 24 (previously presented) - The composition of claim 10 wherein thrombin isolated from the composition has a clotting time of about twenty to about thirty seconds and is stable for up to about 150 minutes.

Claim 25 (previously presented) - The composition of claim 10 wherein thrombin isolated from the composition has a clotting time of about three to about four seconds and is stable for up to about 360 minutes.

Claim 26 (previously presented) - The composition of claim 10 wherein said composition is prepared in a glass container.

Claim 27 (previously presented) - The composition of claim 10 wherein thrombin isolated from the composition is filtered during isolation.

Claim 28 (previously presented) - The composition of claim 10 wherein thrombin isolated form the composition is diluted with saline to alter the clotting time.

Claim 29 (previously presented) - The composition of claim 19 wherein ethanol is present at 13.6% by volume and calcium ions are present at 0.023 μM.

Claim 30 (previously presented) - The composition of claim 19 wherein ethanol is present at a concentration between about 8% and about 18% by volume.

Claim 31 (previously presented) - The composition of claim 19 wherein calcium ions are present at a concentration between about 0.011 μM and about 0.045 μM.

Claim 32 (previously presented) - The composition of claim 19 wherein ethanol is present at 13.6% by volume.

Claim 33 (previously presented) - The composition of claim 19 wherein calcium ions are present at 0.023 μM.

Claim 34 (previously presented) - The composition of claim 19 wherein thrombin isolated from the composition has a clotting time of about five seconds or less and is stable for more than 15 minutes.

Claim 35 (previously presented) - The composition of claim 19 wherein thrombin isolated from the composition has a clotting time of about five seconds or less and is stable for about 240 minutes or greater.

Claim 36 (previously presented) - The composition of claim 19 wherein thrombin isolated from the composition has a clotting time of about twenty to about thirty seconds and is stable for up to about 150 minutes.

Claim 37 (previously presented) - The composition of claim 19 wherein thrombin isolated from the composition has a clotting time of about three to about four seconds and is stable for up to about 360 minutes.

Claim 38 (previously presented) - The composition of claim 19 wherein said composition is prepared in a glass container.

Claim 39 (previously presented) - The composition of claim 19 wherein thrombin isolated from the composition is filtered during isolation.

Claim 40 (previously presented) - The composition of claim 10 wherein thrombin isolated form the composition is diluted with saline to alter the clotting time.

EXHIBIT C

Exhibit C

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Philip H. Coelho, et al.	Art Unit:	1653
Serial No.:	09/709,237	Examiner:	Ms. Chih-Min Kam
Filed:	November 10, 2000		
For:	Apparatus and Method of Preparation of Stable, Long Term Thrombin From Plasma and Thrombin Formed Thereby		

DRAFT AMENDED CLAIMS

Claims 1-9 (cancelled)

Claim 10 (currently amended) - A thrombin composition ~~free of fibrin clots,~~ consisting essentially of:

Plasma;

Ethanol (EtOH) between 8 and 18 percent; and

$CaCl_2$.

Claim 11 (previously presented) - The composition of claim 10 wherein ethanol is present at 13.6% by volume and $CaCl_2$ is present at 0.023 μM.

Claim 12 (withdrawn) – A method for preparing thrombin comprising:

obtaining plasma;

adding only ETOH and $CaCl_2$ to the plasma, forming a composition;

agitating the composition;

filtering the composition of particulate, thereby passing the thrombin through the filter.

Claim 13 (cancelled)

Claim 14 (cancelled)

Claim 15 (previously presented) - The composition of claim 10 wherein $CaCl_2$ is present at a concentration between about 0.011 μM and about 0.045 μM.

Claims 16-18 (cancelled)

Claim 19 (currently amended) - A thrombin composition ~~free of fibrin clots,~~ consisting essentially of:

plasma;

ethanol (EtOH) between 8 and 18 percent; and

a source of calcium ions.

Claim 20 (previously presented) - The composition of claim 10 wherein ethanol is present at 13.6% by volume.

Claim 21 (previously presented) - The composition of claim 10 wherein $CaCl_2$ is present at 0.023 μM.

Claim 22 (previously presented) - The composition of claim 10 wherein thrombin isolated from the composition has a clotting time of about five seconds or less and is stable for more than 15 minutes.

Claim 23 (previously presented) - The composition of claim 10 wherein thrombin isolated from the composition has a clotting time of about five seconds or less and is stable for about 240 minutes or greater.

Claim 24 (previously presented) - The composition of claim 10 wherein thrombin isolated from the composition has a clotting time of about twenty to about thirty seconds and is stable for up to about 150 minutes.

Claim 25 (previously presented) - The composition of claim 10 wherein thrombin isolated from the composition has a clotting time of about three to about four seconds and is stable for up to about 360 minutes.

Claim 26 (previously presented) - The composition of claim 10 wherein said composition is prepared in a glass container.

Claim 27 (previously presented) - The composition of claim 10 wherein thrombin isolated from the composition is filtered during isolation.

Claim 28 (previously presented) - The composition of claim 10 wherein thrombin isolated form the composition is diluted with saline to alter the clotting time.

Claim 29 (previously presented) - The composition of claim 19 wherein ethanol is present at 13.6% by volume and calcium ions are present at 0.023 μM.

Claim 30 (cancelled)

Claim 31 (previously presented) - The composition of claim 19 wherein calcium ions are present at a concentration between about 0.011 μM and about 0.045 μM.

Claim 32 (previously presented) - The composition of claim 19 wherein ethanol is present at 13.6% by volume.

Claim 33 (previously presented) - The composition of claim 19 wherein calcium ions are present at 0.023 μM.

Claim 34 (previously presented) - The composition of claim 19 wherein thrombin isolated from the composition has a clotting time of about five seconds or less and is stable for more than 15 minutes.

Claim 35 (previously presented) - The composition of claim 19 wherein thrombin isolated from the composition has a clotting time of about five seconds or less and is stable for about 240 minutes or greater.

Claim 36 (previously presented) - The composition of claim 19 wherein thrombin isolated from the composition has a clotting time of about twenty to about thirty seconds and is stable for up to about 150 minutes.

Claim 37 (previously presented) - The composition of claim 19 wherein thrombin isolated from the composition has a clotting time of about three to about four seconds and is stable for up to about 360 minutes.

Claim 38 (previously presented) - The composition of claim 19 wherein said composition is prepared in a glass container.

Claim 39 (previously presented) - The composition of claim 19 wherein thrombin isolated from the composition is filtered during isolation.

Claim 40 (previously presented) - The composition of claim 10 wherein thrombin isolated form the composition is diluted with saline to alter the clotting time.

TRANSMISSION VERIFICATION REPORT

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Attn: Ms. Chih-Min Kam

CERTIFICATION OF FACSIMILE TRANSMISSION

I hereby certify that the following in re Serial No. 09/709,237, is being facsimile transmitted to the Patent and Trademark Office on the date shown below:

(1) Declaration of Houria I. Hassouna (6 pgs);

(2) Exhibit A (15 pgs);

(3) Exhibit B (5 pgs); and

(4) Exhibit C (5 pgs).

Please note that the signature page for the Declaration of Ms. Hassouna has been sent twice; a blank page for clarity purposes, and the signed page which has been degraded by facsimilie.

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